Section II. REMARKS

Restriction/Election

In the March 4, 2009 Office Action, the Examiner imposed a restriction requirement under 37 CFR 1.499, wherein applicants are required to elect a single invention from the list below:

Group I:	claims 1-8, drawn to a method for obtaining a singular cell model comprising more than one adenoviral vector comprising an ectopic DNA sequence that codes for a different Phase I drug or a Phase II drug enzyme;
Group II:	claim 9, drawn to a human cell model obtained by the method of Group I;
Group III:	claim 11, drawn to a method for studying the metabolism, pharmacokinetics, potential idiosyncratic hepatoxicity, and/or potential medicament interactions of a drug with the human cell model obtained in Group I; or
Group IV:	claim 13, drawn to a method to confer to any cell line the capacity to metabolize xenobiotics in a controllable manner by means of a set of more than one adenoviral expression vectors selected from the group consisting of Phase I enzymes, Phase II enzymes and cytochrome P450 reductase.

Applicants elect, with traverse, Group II (claim 9) drawn to a human cell model. Applicants have also introduced claims 14-20 as dependant from claim 9 and thus, said claims also form part of group II. The newly added claims do not add subject-matter to the application as filed.

Applicants respectfully disagree with the need for restriction in this case. The European Patent Office (EPO), acting as the International Search Authority (ISA) of international patent application PCT/EP2005/007535, from which this U.S. patent application derives, has already reviewed the unity of invention issue. In fact, the International Search Report (ISA) concluded that the set of claims met the requirement of unity of invention since no objections on said requirement were issued. The documents cited on the ISA have already been forwarded to the US Patent and Trademark Office (USPTO) for the purpose of national stage entry of said international patent application. The ISA has found that the present invention relates to "one invention only or to a group of inventions so linked as to form a single general inventive concept" (PCT Rule 13.1).

The ISA has applied the PCT rules to the present invention without finding a lack of unity of invention. The USPTO cannot apply now a different standard to the present invention than the ISA.

That said, the Examiner is of the opinion that the technical feature linking the inventions of groups I-IV does not constitute a special technical feature under PCT Rule 13.2, as it does not define a contribution over the prior art. Applicants respectfully disagree for the following reasons.

The technical feature linking the inventions of groups I-IV of the present invention is a human cell model capable of reproducing *in vitro* the metabolic idiosyncrasy of humans characterized in that said cells are human cells of hepatic origin expressing reductase activity and transformed with a set of more than one recombinant adenoviral expression vectors, wherein each expression vector comprises an ectopic DNA sequence that codes for a different Phase I or Phase II drug biotransformation enzyme. Applicants consider that said technical feature of the invention defines a contribution over the prior art for the following reasons.

Brimer et al. (*Pharm. Res. 2000 U.S.*, 17 (2000) 803-810) (hereinafter Brimer) discloses the use of human hepatocytes as a tool for studying toxicity and metabolism. Brimer also discloses at pages 307-308 human hepatocyte cells transduced with adenovirus expression of transcription factors, resulting in an increase of CYP3A4 mRNA expression.

The cells of present claim 9 differ from those described by Brimer in that the adenoviral expression vectors used comprise ectopic DNA sequences that code directly for different drug biotransformation enzymes, and not for their transcription factors.

Brimer discloses LLC-PK1, L-MDR1 and Caco-2 cell lines that have been transduced with an adenoviral vector comprising human CYP3A4 cDNA. For example, both CYP3A4 and NADPH cytochrome P 450 reductase are expressed with adenovirus. However, the NADPH 450 reductase is not a "Phase I or Phase II drug biotransformation enzyme" but rather an electron donor protein for several oxygenase proteins, necessary for the metabolic activity of CYPs. It does not itself metabolize the drug.

The Examiner stated that:

> "Brimer et al. discloses adenoviral transduction of various cell lines with more than one vector encoding Phase I biotransformation enymes, CYP3A4 and NADPH P450 reductase, but not hepatic cells. Gomez Lechón et al. . . . discloses a method of adenoviral transduction of hepatic cells with a vector encoding Phase I drug biotransformation enzyme. Therefore it would have been obvious to

the one skill in the art to to choose hepatic cells as alternatives to the cells of Brimer et al, therefore claim 9 lacks an inventive step."

Applicants disagree. The problem to be solved in the present invention is to obtain a suitable alternative model **based on human hepatocytes** for the study of xenobiotic metabolism. The Brimer cell model specifically refers to intestinal cells (where CYP activity is not significant but drug transport is a major contributor), thus, the use of hepatic cells described in Gomez-Lechón et al. (*Current Drug Metabolism*, 2003, 4, 292-312) (hereinafter Gomez-Lechón) would not have been appropriate to compliment the teachings of Brimer, since the aim of Brimer was to generate a cell model of intestinal cells.

Gomez-Lechón discloses a cell model for drug metabolism in humans consisting of human hepatocytes in primary culture. These cells usually come from few donors and show high interindividual differences. In order to solve the problem, the authors of Gomez-Lechón suggest modulating the expression of the CYP enzymes by transducing hepatocytes with adenoviral vectors encoding for the so-called LETFs (liver-enriched and ubiquitous regulatory factors) which are transcription factors which are involved in the regulation of one or more of CYP enzymes (see, e.g., page 307, 2nd col., first paragraph). Thus, two vectors encoding for two transcription factors (HNF3-γ, C/EBP-α) were constructed in an attempt to regulate CYP genes expression in non metabolic competent cell lines. Although the mRNA levels of some CYPs increased (e.g., upwards of 60-fold), no correlation was found between mRNA levels and CYP activity. In the best case scenario 1000-fold less CYP3A4 activity was found when compared with human activities. Consequently, the teaching from Gomez-Lechón is that the infection with key transcription factors adenovirus alters the expression but not the function of hepatic drug-metabolizing enzymes.

In contrast, the cell model of claim 9 differs from Gomez-Lechón in that the expression of the different CYP enzymes is modulated by transducing the hepatocytes with a plurality of different adenoviral vectors, each containing the coding sequence of a different CYP isoform. Accordingly, the technical effects associated with said difference include:

(i) the expression of individual CYP isoforms can be regulated more precisely because the transduction of the hepatocytes with a given adenoviral vector results in the expression of a single CYP isoform (see, e.g., example 2 of the instant application), whereas the overexpression of a transcription factor triggers the simultaneous modulation of multiple target genes including one or more of the target CYP genes as well as other unrelated genes. Moreover, the combined effect of CYP adenovirus mixture allow to control

different CYP activities as desired, providing the possibility of mimicing the *in vivo* human behaviour of hepatocytes (see, figure 1 below). Additionally, the cell model of claim 9 has normal human hepatic CYP activities and even higher activities can be reached. This feature of the cells of claim 9 permits, for instance, the possibility of drug induction.

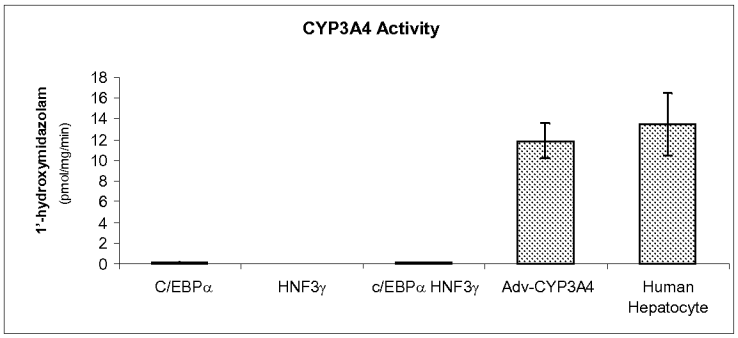

Figure 1. Comparative bar chart showing the different CYP3A4 activities in HepG2i transfected with C/EBP□ vector (10 MOI), HepG2i transfected with HNF3γ vector (10 MOI), HepG2 transfected with a mixture of both C/EBPα and HNF3γ vector, HepG2 transfected with Adv-CYP3A4 vector and human hepatocyte culture. CYP3A4 activity was measured as midazolam hydroxylation by HPLC-MS/MS.

(ii) a linear correlation between the amount of adenoviral vector added and CYP activity. In Figure 2 (see below) an additional feature of the cell model of the invention is shown, which is the possibility of achieving even higher enzymatic activities than that found in human hepatocyte cultures. This fact allows the model to mimic different idiosyncratic situations, e.g., enzymatic induction by drug co-administration, polymorphism, etc. Thus the transduction of the cells with adenoviral vectors containing the CYP cDNA results in a corresponding increase in the CYP activity (see figure 2), whereas the transduction of cells with adenoviral vectors expressing transcription factors results in the over-expression of the mRNA coding for the CYP isoform (see, e.g., Gomez Lechón, figure 7, p. 307, 2nd col., lines 32-34) without the corresponding increase in activity.

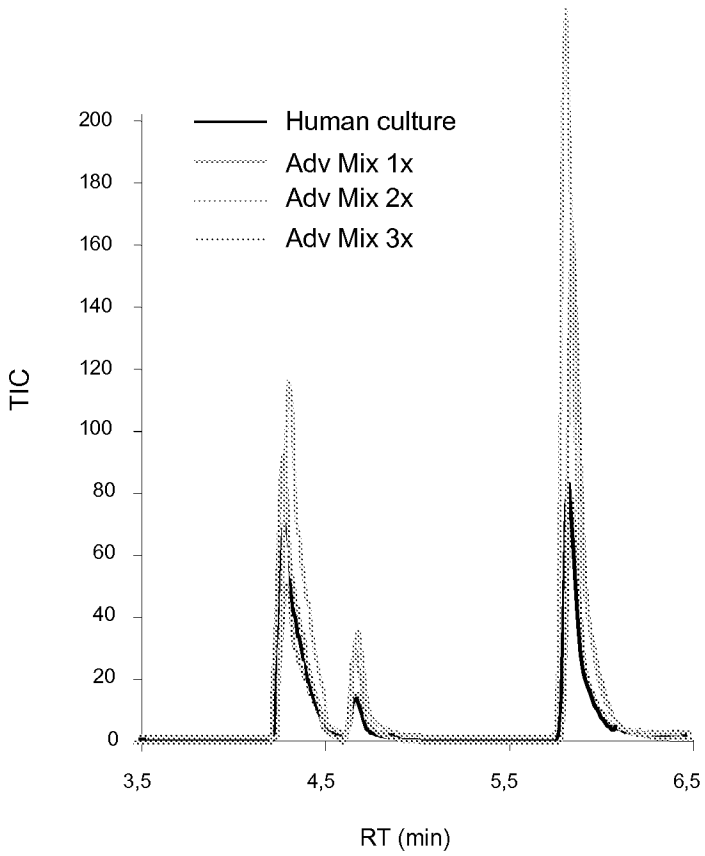

Figure 2. Reconstructed total ion chromatograms of HPLC/MS-MS of the CYP 2E1, 3A4 and 2C9 activities profile. Infections with increasing MOI allow controlled over expression of CYP activities, reaching even higher enzymatic activities than that found in human hepatocyte culture.

When considering Brimer in combination with Gomez-Lechón, as proposed by the Examiner, the solution provided in the present application could not have been derived in an obvious manner for at least the following reasons:

- Brimer is concerned with achieving good expression levels of a particular CYP polypeptide and of a transport protein in polarized cells and thus, there is no reason why the skilled person would consider its teaching at all when looking for a method to achieve precise regulation of activity levels in hepatocytes.

- Brimer mentions that different cell lines are transduced with different efficiency, in particular, LLC-PK1 were superior to human Caco-2 cells in AdV infectivity and expression of catalytically active CYP3A4 (see Brimer, page 809, last paragraph), teaching away from the approach to reproduce the human metabolic idiosyncrasy in hepatocytes.

- the functional activity (measured by the formation of hydroxymidazolam) of the modified Caco-2 cells obtained in Brimer (Caco-2/Ad3A4/AdRed) is substantially different from normal human activities as can be seen in table 1 of Brimer (please note the difference in magnitude of the units). It is much lower than that of human liver microsomes (see Brimer, page 808, first paragraph).

- Brimer is silent about the interactions that can be expected if more than one enzyme is introduced, and that can affect the activity of each of them.

Thus, it is submitted that the cell model which is considered as the technical feature linking the inventions of groups I-IV would not have been derived in an obvious manner by a person skilled in the art from Gomez-Lechón when taken together with Brimer. Consequently, the technical feature involves an inventive step and therefore it does constitute a special technical feature linking the inventions of groups I-IV. Withdrawal of the restriction requirement under 37 CFR 1.499 is respectfully requested.

If the restriction requirement nonetheless is made final, applicants alternatively request rejoinder of method claims 1-8 under the provisions of MPEP §821.04 upon confirmation of allowable subject matter of the Group II claims 9 and 14-20.

Such rejoinder would be fully proper under these circumstances for the following reasons.

When an application as originally filed discloses a product and the process for making and/or using such product, and only the claims directed to the product are presented for examination, when a product claim is found allowable, applicant may present claims directed to the process of making and/or using the patentable product for examination through the rejoinder procedure in accordance with MPEP §821.04, provided that the process claims depend from or include all the limitations of the allowed product claims.

In the present application the elected claims 9 and 14-20 are directed to a human cell model capable of reproducing *in vitro* the metabolic idiosyncrasy of humans characterized in that said cells are human cells of hepatic origin expressing reductase activity and the non-elected method claims 1-8 are directed to a method of obtaining said cell model. Consistent with the provisions of the MPEP

§821.04, when the product claims 9 and 14-20 are found allowable, the withdrawn method of use claims 1-8 may be rejoined for examination.

In the event that the Examiner does not withdraw the restriction requirement, applicants reserve the right to take any other measure as deemed appropriate, such as the filing of a divisional application, in order to protect the subject-matter of the non-elected claims.

In addition, in the March 4, 2009 Office Action, applicants were required to elect a single species from claims 14-17. In response, applicants elect oxygenases from claim 14, monooxygenases dependent on CYP450 from claim 15, DNA sequences transcribed in the sense mRNA of CYP450 isoenzymes from claim 16, and DNA sequences transcribed in the sense mRNA of CYP3A4 from claim 17. Applicants acknowledge the Examiner's indication that upon allowance of a generic claim, applicants will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim.

Petition for Extension of Time/Fees Payable

Applicants hereby petition for a four (4) month extension of time, extending the deadline for responding to the March 4, 2009 Office Action from April 4, 2009 to August 4, 2009. The fee of $865.00 specified in 37 CFR §1.17(a)(4) for such four (4) month extension is hereby enclosed.

Seven (7) claims have been added, bringing the total number of pending claims to twenty (20), three (3) of which are independent. As such, no added claims fee is due at this time.

The total fee of $865.00 is being paid by Electronic Funds Transfer. Authorization is hereby given to charge any deficiency in applicable fees for this response to Deposit Account No. 13-4365 of Moore & Van Allen PLLC.

Conclusion

If any additional issues remain, the Examiner is requested to contact the undersigned attorney at (919) 286-8000 to discuss same.

Respectfully submitted,

MOORE & VAN ALLEN PLLC

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By: _____

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